Amendments to the Specification:

Please replace the title with the following amended title: METHOD OF PRODUCING AN HIV-1 IMMUNE RESPONSE.

Please add the following new paragraph after the title, on page 1:

This application is a divisional application of Application No. 09/674,674, which was PCT filed on March 2, 2000.

Please replace the paragraph starting at page 1, line 14 with the following amended paragraph:

There is an urgent need to control the global epidemic of HIV infection and the development of a vaccine against HIV is one of the major objectives in AIDS research. In general vaccines should activate antigen presenting cells, overcome genetic restriction in T-cell responses and generate T- and B-memory cells. The variability or the viral population poses a further difficulty in obtaining an effective HIV vaccine. A ~~break-through~~ breakthrough in the ongoing attempts to develop a vaccine against AIDS has so far not been reported. It is now generally accepted that an induction of antigen-specific humoral and cell-mediated immunity is crucial for a development of an effective prophylactic and therapeutic vaccine. All three arms of the immune system including neutralizing antibodies; CD8+CTL and T-helper-1 (TH1) cells might be required for protective immunity to HIV. It is known that CTL can clear other viral infections (Ada, Immunol. Cell Biol., 72:447-454, 1994) and that CTL can lyse infected targets early in infection before viral progeny can be produced and released by cell lysis, Ada et al.,

supra. The focus has been on selection of antigens as well as on design and evaluation of different adjuvances. The antigens used in different *in vitro* and *in vivo* studies have all been ~~all~~ from crude proteins to various synthetic peptides, mainly from gp160 and to some extent from p24. A large number of studies have been done on the V3 loop of gp120. Induction of both B- and T-cell responses have been ~~observed;~~ observed; however, it has been reported from an *in vitro* study that a peptide from the conserved region of gp41 ~~have~~ has indicated infection enhancement (Bell S.J., et al., Clin. Exp. Immunol., 87 (1): 37-45, (January 1992).

Please replace the paragraph starting at page 2, line 1 with the following amended paragraph:

Naturally occurring HIV sequences in vaccine candidates are not capable of stimulating a stable immune response due to the ~~viruses~~ virus's inherent ability to hide by changing the appearance of the epitopes presented on the cell surface of infected cells. The immune system is fooled ~~to believe~~ into believing that a particular amino acid sequence is relevant when in fact the amino ~~acids~~ acid of importance is hidden.

Please replace the paragraph starting at page 2, line 19 with the following amended paragraph:

Johnson R.P., et al., The Journal of Immunology, Vol. 147, p. 1512-1521, No. 5, September 1, 1991 describe an analysis of the fine specialty of gag-specific CTL-responses in three HIV-1 seropositive ~~individuals;~~ individuals. The ~~the~~ gag-specific CTL-responses were found to be mediated by CD3+CD8+ lymphocytes which are HLA class I restricted.

Please replace the paragraph starting at page 3, line 4 with the following amended paragraph:

EP 0 230 222, EP 0 270 114, DE 37 11 016 and GB 2 188 639 all in the name of F. Hoffmann-La Roche & Co. Aktiengesellschaft concern recombinant expression and purification of an HTLVIII Gag/Env gene protein or ~~fusionproteins~~ fusion proteins. The proteins consisting of native sequences can be purified to homogeneity and used as a basis for diagnostic tests for detection of antibodies against viruses associated with AIDS. The gag/env protein may also be formulated for use as a vaccine for protection against AIDS through prophylactic immunization.

Please replace the paragraph starting at page 3, line 12 with the following amended paragraph:

From a diagnostic and therapeutic point of view, the major ~~problems~~ problem with using p24 as part of an assay or therapy is associated with the high number of epitopes on p24 which stimulates production of a large number of antibodies with poor specificity, which through repeated boostering on potential mutated sequences can create autoantibodies (Autoantibodies to the alfa/beta T-cell receptors in HIV infection; dysregulation and mimicry. Lake D.F., et al., Proc. Nalt. Acad. Sci. USA, (23) : 10849-53, Nov. 8 1994). Further, it is reported that the p24 antibody titer does not reach the same high levels as for the envelope proteins (gp120 and gp41). Normally antibodies to p24 are developed in the early phase of the infection, but the titer is fairly quickly stabilized after the initial infection period. Later the p24 titer ~~is~~ gradually ~~decreasing~~ decreases while the opposite happens with gp160. These findings

can also be seen in relation to recent reports stating that cytotoxic T-cell activity is antagonized by naturally occurring HIV-1 gag variants (Klenerman, P., et al., Nature, 2:369 (6479), p. 355, 2 June 1994). This can be one of the reasons why a rapid stabilization of the p24 titer is seen and why it later starts to decrease.

Please replace the paragraph starting at page 4, line 1 and ending at line 11 with the following amended paragraph:

The initial work was based on one epitope which was published by Korber B., et al., Human Retroviruses and AIDS 1997 Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM. The amino acid sequence of this epitope (203-222) was:

K A L G P G A T L E E M M T A C Q G V G (SEQ ID NO: 26)

R R M R T K S I K D L S S S R R (SEQ ID NO: 27)

G V R V (SEQ ID NO: 28)

S A A (SEQ ID NO: 29)

S E (SEQ ID NO: 30)

Q Q (SEQ ID NO: 31)

Please replace the paragraph starting at page 4, line 13 and ending at line 28 with the following amended paragraph:

The one letter as well as the three letter codes defining the amino acids in the sequences given throughout this specification are in accordance with International standards and

given in textbooks, for instance Lehninger A.L., «Principles of Biochemistry», Worth Publishers Inc., New York, 1982. The ~~aminoacids~~ amino acids given below the head sequence represent the natural variation of the sequence. An initial study of a sequence containing this modified epitope was conducted on the sequence:

ANPDCKQILKSLGPGATLEEXXTACQGVG - NH_2 (SEQ ID NO: 32)

wherein X indicates 2-aminohexanoic acid, and the cysteine residues are in an oxidized state, i.e. are forming an intrachain disulphide bridge. The results (unpublished) from studies using this peptide as part of a diagnostic kit showed that the specificity became 87% (n=279) on a preselected panel of African sera. The sensitivity was surprisingly 100% on a panel of HIV-1 positive sera including HIV-1 subtype O sera, which is quite different from the other subtypes.

Please replace the paragraph starting at page 4, line 30 and ending at page 5, line 2 with the following amended paragraph:

In order to improve specificity, i.e. define the amino acids which contribute to a pure non-crossreacting antibody response, a similar study was applied to a significantly shorter and further modified peptide:

L I W G A T C Q E H X T A C Q G V G - NH_2 (SEQ ID NO: 33)

wherein X has the above-mentioned meaning and the cysteine residues are forming an intrachain disulphide bridge.

Please replace the paragraph starting at page 5, line 6 and ending at line 14 with

the following amended paragraph:

The results from this study showed that the specificity of the assay increased to 96%, and (n=293) which is similar to the specificity obtained in the assay without using the p24 peptide. With a specificity of 87% to the assay where the first peptide was included, it would be likely that the peptide would induce an immune response to more than one epitope since it was recognized by unspecific antibodies, if it was used as a vaccine candidate. The latter, however, shows that the peptide sequence is picking up an immune response which is unique to HIV-1. Consequently, if a sequence based on this is used as an antigen in a vaccine candidate, it would most likely boost ~~an~~ a unique immune response to HIV-1.

Please replace the paragraph starting at page 5, line 16 and ending at page 6, line 10 with the following amended paragraph:

To further increase the number of T-cell epitopes and reduce the probability for development of escape mutants, three additional peptide sequences were based on the following three sequences from residues 264-284, 253-271 and 166-186, respectively, published in Human Retroviruses and AIDS 1997; A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences, Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos:

R W I I L G L N K I V R M Y S P T S I L D (SEQ ID NO: 34)

K G V V M M K C V G E (SEQ ID NO: 35)

D M V V Q I G (SEQ ID NO: 36)

S (SEQ ID NO: 37)

A (SEQ ID NO: 38)

N N P P I P V G E I Y K R W I I L G L (SEQ ID NO: 39)

S Q A V K D M L R K G M V M (SEQ ID NO: 40)

G G S N K V D V V (SEQ ID NO: 41)

H G T (SEQ ID NO: 42)

A (SEQ ID NO: 43)

P (SEQ ID NO: 44)

and

P E V I P M F S A L S E G A T P Q D L N T (SEQ ID NO: 45)

R I T T T L T E A D I S Y N I Y M (SEQ ID NO: 46)

L N A L V H V I (SEQ ID NO: 47)

M L A (SEQ ID NO: 48)

V (SEQ ID NO: 49)

Several modified peptides have been synthesized in order to determine unique sequences which are both specific and sensitive towards HIV-1.

Please replace the paragraph starting at page 6, line 15 and ending at line 19 with the following amended paragraph:

The peptides according to the invention ~~are originating~~ originate from the four different conserved areas of the HIV-1 core protein p24 which are described above, having the properties of maintaining the uniqueness (sensitivity and specificity) of the HIV-1-epitope.

Further, the new peptides according to the invention possess no recognized cytotoxic T lymphocyte (CTL) antagonistic effect and shall have at least one potential CTL epitope.

Please replace the paragraph starting at page 10, line 11 with the following amended paragraph:

The C- and N-terminals ends of the peptide sequences could deviate from the natural sequences by modification of the terminal NH_2-group and/or ~~COOH-group, they~~ COOH-group. They may, for instance, be acylated, acetylated, amidated or modified to provide a binding site for a carrier or another molecule.

Please replace the paragraph starting at page 10, line 16 with the following amended paragraph:

The peptides according to the invention ~~are consisting~~ consist of 6 to 50 amino acids, preferably between 10 and 30 amino acids. They ~~are covering~~ cover all natural ~~variation~~ variations of amino acids in the identified positions.

Please replace the paragraph starting at page 10, line 26 with the following amended paragraph:

Examples of carriers that can be used for ~~e.g.~~ diagnostic purposes, for example, are magnetic beads or latex of co-polymers such as styrene-divinyl benzene, hydroxylated styrene-divinyl benzene, polystyrene, carboxylated polystyrene, beads of carbon black, non-activated or polystyrene or polyvinyl chloride activated glass, epoxy-activated porous magnetic

glass, gelatine or polysaccharide particles or other protein particles, red blood cells, mono- or polyclonal antibodies or fab fragments of such antibodies.

Please replace the paragraph starting at page 11, line 28 with the following amended paragraph:

Another approach in order to enhance the stimulation and absorption in, for instance, the intestine is to administer the peptides of the ~~invention,~~ invention with small peptides such as di- tri- or tetra peptides. These peptides can be administered in addition to or in combination with the peptides of the invention. Preferably the peptides are administered together with the tripeptide YGG, consisting or amino acids in the D- or L-forms, preferably in the D-form.

Please replace the paragraph starting at page 12, line 1 with the following amended paragraph:

Recent approaches to non-parenteral delivery of vaccines, for instance, via mucosa ~~includes;~~ include: gene fusion technology to create non-toxic derivatives of mucosal adjuvants, genetically inactivated antigens with a deletion in an essential gene, coexpression of an antigen and a specific cytokine that is important in the modulation and control of a mucosal immune responses, and genetic material itself that would allow DNA or RNA uptake and its endogeneous expression in the host's cells.

Please replace the paragraph starting at page 12, line 8 with the following amended paragraph:

One approach for developing durable responses where cell-mediated immunity is ~~required,~~ required is to vaccinate with plasmid DNA encoding one or more specific antigen(s).

Please replace the paragraph starting at page 12, line 11 with the following amended paragraph:

In order to protect against HIV infection, vaccines should induce both mucosal and systemic immune responses and could be administered by any convenient route, parenterally or non-parenterally, such as ~~subcutanously~~ subcutaneously, ~~intracutanously~~ intracutaneously, intravenously, intramuscularly, perorally, mucosally or intranasally, for example.

Please replace the paragraph starting at page 12, line 16 with the following amended paragraph:

In a preferred embodiment, ~~of~~ the vaccine according to the present invention ~~it~~ comprises antigens containing the peptides of the SEQ ID NO : 1, 4, 9 and ~~15, more preferred~~ 15. More preferably the peptides occur in the ratio 1:1:1:1.

Please replace the paragraph starting at page 14, line 3 with the following amended paragraph:

All peptide derivatives prepared in the Examples given below were synthesized on a Milligen 9050 Peptide Synthesizer using a standard program. The resin used was Tenta Gel P

RAM with a theoretical loading of ~~0,20~~ 0.20 meq/g (RAPP POLYMERE GmbH, Tübingen). The final product of the synthesis was dried *in vacuo* overnight. The peptide was then cleaved from the resin by treatment with 90% trifluoroacetic acid in the presence of ethandithiol (5%) and water (5%) as scavengers (~~1,5~~ 1.5 hours at RT). Then the resin was filtered and washed on filter with additional trifluoroacetic acid (100%) (2 x 20 ml). The combined filtrates were evaporated *in vacuo* (water bath at RT) and the residue was triturated with ethyl ether (200 ml) and the precipitated product filtered off. The solid was promptly dissolved on filter with glacial acetic acid (100 ml) and added to ~~1,5~~ 1.5 l of 20% acetic acid in methanol and treated with ~~0,1~~ 0.1 M solution of iodine in methanol until a faint brown colour remained. Then Dowex 1 x 8 ion exchange in acetate form (15g) (Bio-Rad, Richmond, CA) was added and the mixture filtered. The filtrate was evaporated and the residue freeze-dried from acetic acid. The product was then purified by reversed phase liquid chromotography on a column filled with Kromasil® 100 - 5 C8 (EKA Nobel, Surte, Sweden) in a suitable system containing acetonitrile in ~~0,1~~ 0.1 % trifluoroacetic acid water solution. The samples collected from the column were analyzed by analytical high performance liquid chromotagraphy (HPLC) (Beckman System Gold, USA) equipped with a Kromasil® 100 - 5 C8 Column (EKA Nobel, Surte, Sweden). Fractions containing pure substance were pooled, the solvent was evaporated and the product freeze-dried from acetic acid. The final HPLC analysis was performed on final product, and the structure of the peptide was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Please replace the paragraph starting at page 18, line 24 with the following amended paragraph:

The peptide sequences of ~~the~~ Examples 1 and 3 were linked via an oxidation step to form a dipeptide wherein the cysteine residues formed a disulphide bridge. The bridge was formed in either of two ways[[;]] :

A) Oxidation with I_2. Equals amounts of the peptides were dissolved in acetic acid methanol (1:4) and 0.1 M I_2 in methanol was added yielding a mixture of the dimer.

or

B) Oxidation via [Cys(Spy)^{16}]-SEQ ID NO : 2. ~~2,3~~ 2.3 mM of the peptide of SEQ ID NO : 2 dissolved in 2 M AcOH (aq) and 2-propanol (1:1) was treated with 2,2 dithiodipyridin (3eqv) to yield [Cys(Spy)^{16}]-SEQ ID NO : 2. Equal amounts of [Cys(Spy)^{16}]-SEQ ID NO : 2 and peptide of SEQ ID NO : 5 were dissolved in 10 mM NH_4Oac (aq pH=~~6, 5~~ 6.5) and methanol (5:2) to yield the dimer of SEQ ID NO : 21.

Please replace the paragraph starting at page 19, line 18 with the following amended paragraph:

An antigen solution or suspension is mixed with equal parts of Freund's adjuvant of Behring, complete or incomplete, and is then finely emulsified by being drawn up into, and ~~vigurously~~ vigorously pressed out of, an injection syringe, or with a homogenator. The emulsion should remain stable for at least 30 minutes. The antigen-adjuvant emulsions is best injected subcutaneously as a depot.

Please replace the paragraph starting at page 19, line 31 with the following amended paragraph:

Toxicity studies were performed in mice and rats on the peptide composition of the vaccine in Example 14. The ~~mouse was~~ mice were selected for the study to provide comparative data from a second commonly used rodent species. The test substance was a mixture of four peptides supplied ~~as~~ in one vial containing lyophilised material for reconstitution with physiological saline, and dose levels were expressed in terms of total peptide load. The individual peptides ~~was~~ were present in the ratio 1:1:1:1, giving dose levels of each peptide of 0.0075 mg/kg body weight, 0.075 mg/kg body weight and 0.75 mg/kg body weight, which are up to 500 fold the intended human dose. The test animals were divided into four groups of ten animals each (five males and five females); a saline control group and groups for low, intermediate and high doses. The test composition was administered once, by intravenous infusion into a tail vein at a dose rate of 3 ml/minute. The animals were killed at day 15 and 16 by intraperitoneal injection of sodium pentobarbitone.

Please replace the paragraph starting at page 21, line 26 with the following amended paragraph:

The polypeptides of the invention can be used in a combination of at least one peptide selected from each group of sequences, SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 9 and SEQ ID NO : 15 to form antigens and the the active principle of a proplylactic or therapeutic vaccine intended to provide protection against the human immunodeficiency virus type 1 (HIV-1). The vaccine may include compounds having beneficial effects in protecting or stimulating the host's immune system (human being or vertebrate animal) for instance interleukins, interferons, granulocyte macrophage growth factors, haematopoietic growth factors or similar.

Preferably the vaccine composition further ~~contain~~ contains an adjuvant or vehicle, more ~~preferable~~ preferably the adjuvant or vehicle is Monophosphoryl Lipid A (MPL ®) possibly with alum, Freund's adjuvant (complete or incomplete) or aluminum hydroxyd. The optimal amount of adjuvant/vehicle will depend on the type(s) which is chosen.

Please replace the paragraph starting at page 22, line 5 with the following amended paragraph:

The peptide or vaccine formulation can be freeze-dried prior to storage. The vaccine may be stored preferably at low temperature, in ampoules containing one or more dosage units, ready for use. A typical dosage unit of the peptide according to the invention is within the concentration range: 1μg-1mg per kg bodyweight, preferably within 2 μg-0.15 mg per kg bodyweight. Persons skilled in the art will appreciate that a suitable dose will depend on the body weight of the ~~pasient~~ patient, the type of disease, severity of condition, administration route and several other factors. The vaccine might be administered up to twelve times and through injection, typically it will be administered about three times. In preparation of an injection solution the peptides are dissolved in sterile sodium chloride solution at a final concentration of 1 mg/ml per peptide and ~~0,9~~ 0.9 % sodium chloride. Typically an injection volume is 100 μl to 200 μl (2 x 100 μl). The peptide is preferably co-administered with a suitable adjuvant and/or a granulocyte-macrophage growth factor for instance Leucomax ® «~~Shering~~ Schering Plough». Suitable administration may be ~~intracutane, subcutane~~ intracutaneous, subcutaneous, intravenous, peroral, intramuscular, intranasal, mucosal or any other suitable route. Booster administrations may be required in order to maintain protection. For persons skilled in the art it will be

understood that the vaccine compositions according to the invention are useful not only in the prevention of infection, but also in the treatment of infection.